RESPIRATION OF OOCYTES, UNFERTILIZED EGGS AND FERTILIZED EGGS FROM PSAMMECHINUS AND ASTERIAS

HANS BOREI

Wenner-Gren's Institute for Experimental Biology, University of Stockholm

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1. Introduction

Using Cartesian diver micro-respiration technique Lindahl and Holter (1941) measured the oxygen consumption rate of primary oocytes, mature unfertilized eggs and fertilized eggs of the sea-urchin *Paracentrotus lividus*. They found that the oocyte respiration is markedly higher than that of the unfertilized egg and that it probably exceeds that of the newly fertilized egg. On the other hand Boell, Chambers, Glancy and Stern (1940) stated, in an earlier brief note, that in similar diver measurements the mature unfertilized *Arbacia* egg reveals a higher oxygen consumption and a higher capacity to oxidize p-phenylenediamine than does the immature egg (oocyte).

Because of these two quite divergent results it was considered to be of interest to investigate, and eventually settle the matter, by using a third sea-urchin species. *Psammechinus miliaris* from the Swedish West Coast was chosen. This species offers some special advantages: its spawning period is relatively long; its oocytes can be obtained regularly during practically the whole of the spawning period; and, there are three distinct cytoplasmic maturity stages of the egg, viz., under-ripeness, ripeness and over-ripeness. These maturity stages are characterized by differences in fertilizability, in fertilization membrane appearance and in reaction to hypertonic medium (cf. Runnström and Monné, 1945). Thus it might be possible to follow in detail any gradual alteration in oxygen consumption during the entire egg maturation process. Since it is necessary selectively to pick out the desired cells from the cell mixture, which is extruded from the ovary, the diver technique, which permits the measurement of the oxygen consumption of as few as about a hundred oocytes or resting eggs, will be very suitable. As only a small number of cells are

necessary for each experiment, it is possible to charge any desired parallel diver unit with cells from the same ovary.

Furthermore, it was thought that a comparison between the sea-urchin oocyte or egg respiration and that of the starfish oocyte or egg might throw some light on the general laws of echinoderm egg metabolism. Tang (1931), using a Warburg technique, found that mature and immature (oocytes) *Asterias* eggs used up the same oxygen amount per time unit. Boell, Chambers, Glancy and Stern (1940) arrived at the same result using diver technique, but Brooks (1943) in Warburg experiments found a lower consumption in immature eggs than in mature. In the present paper diver measurements on *Asterias glacialis* oocytes and eggs will be reported.

In addition, diver measurement data concerning the oxygen consumption of the fertilized sea-urchin and starfish egg are given and interpreted. In *Asterias* the respiration is only followed over the first mitosis, but in *Psammechinus* it is measured until some hours after hatching.

Abbreviations used in the text:
Ps.: *Psammechinus miliaris*
Ast.: *Asterias glacialis*
Par.: *Paracentrotus lividus*

2. General Remarks on Material and Methods

2.1. *Animals*

Ps. occurs in two phenotypic varieties: one, called the *Z-form,* is the trivial littoral form; the other, called the *S-form,* is found at greater depths. They differ in size and morphological appearance and, of special interest in this investigation, in the spawning period. The *Z-form* has fertilizable eggs from the middle of June until the middle of July, the *S-form* during July and August. Concerning living conditions, distribution and biology, cf. Lindahl and Runnström (1929) and Borei and Wernstedt (1935).

The *Ps. Z-form* animals were dredged from about 6 m. depth. They were kept in a wire mesh cage immersed in the surface water off the station pier, where temperature and salinity conditions were approximately the same as those at the dredging-locality. The sea-urchins were used for experiments within a few days of being caught.

The *Ps. S-form* sea-urchins were caught at about 20–30 m. depth. They were brought to the station immersed in 32–33 ‰ salinity water in a big Dewar vessel to keep the temperature low. At the station they were transferred to aquaria with running sea water, where the salinity was about 32–33 ‰ and the temperature between 15–17° C. The conditions at the dredging-localities were about the same with regard to salinity, but somewhat lower as regards temperature. The animals were mostly used for experiments on the day of capture; in some rare cases they were not used until the next day.

Ast. was dredged from 30–40 m. depth. The animals were brought to the station and kept there in the manner described above for the *Ps. S-form.* They were invariably used for experiments on the day of capture. Their spawning period falls mainly in May and June.

2.2. *Diver technique*

The technique of Cartesian diver measurements has been described in detail by Holter (1943). Only points of special interest will be mentioned here.

The divers used were of standard type (volume 8–10 μl.) made of Jena Geräte glass ($\phi = 2.412$). They were charged as follows, according to the "Diver charge Type I" of Borei (1948):

Mouth seal: Holter's medium ($\phi_M = 1.325$)
Neck seal I: 0.5 μl. paraffin oil ($\phi_{oi} = 0.87$)
Neck seal II: 0.5 μl. isotonic sodium hydroxide } ($\phi_W \simeq 1.0$)
Bottom drop: 0.8 μl. sea water cell suspension }
(cf. Borei (1948), Figure 1: I).

The cells were extruded from the ovary after this had been removed from the body, sifted through bolting-cloth and washed three times in sea water before being picked up in a braking pipette.

The salinity of the sea water of the cell suspension varied according to the material. For the *Ps. Z-form* water of 24.6 ‰ S was mostly used. This salinity figure approximately equals the medium salinity during the summer months of the surface water off the station pier where the animals were kept. Occasionally higher salinities occur. Thus it was sometimes found more correct to apply water of 27 or 29 ‰ S. For the *Ps. S-form* which lives in water of higher salinity and was therefore kept in the station aquarium sea water; 32–33 ‰ S sea water was used. In the *Ast.* experiments the salinity was 29 ‰ throughout. This salinity is somewhat lower than that on the dredging-localities, but had to be used owing to some temporary trouble with the station sea water pipe-line. All salinities were checked by titrimetric estimations according to Borei (1947). For pH control potentiometric measurements (glass electrode) were employed. Isotonic NaOH solutions for the diver neck seal II were prepared from a stock solution. (From the sea water freezing points tabulated by Knudsen (1903) it can be calculated that 0.365 N NaOH is isotonic to 25 ‰ S sea water.)

The temperature in the experiments was mainly 18° C. For some *Ps.* experiments temperatures between 15–21° C. were employed, owing to the requirements of simultaneous measurements for other investigations. The maximum temperature for normal larval development of *Ps.* was studied by Runnström (1927) and found to be 22° C. *Ast.* belongs to the same species group, the mediterranean-boreal, and is likely to have about the same upper temperature limit for normal development. The temperatures in the experiments are thus well below the critical level.

The number of eggs per diver was 40–50 for *Ast.*, 70–120 for *Ps.* when unfertilized, and 40–50 for *Ps.* when fertilized. These numbers give approximately 12.5, 8 and 9×10^{-3} μl. oxygen consumed per hour respectively in the most crowded divers, *i.e.* δp lies between 1–2 cm. per hour. This rate is best suited to keep the errors of the diver apparatus low (cf. Holter, 1943) and lies, moreover, within the range $3\text{–}18 \times 10^{-3}$ μl. per hour, which Lindahl and Holter (1940) in diver experiments on *Par.* found to be characterized by direct proportionality between number of cells and oxygen consumption. Lindahl and Holter (1940) further found that diver and Warburg experiments which were performed simultaneously gave very consistent results. The same applies to the present diver experiments on unfertilized *Ps.* eggs compared with the Warburg experiments of Borei (1934) (cf. 3.112.2). Thus the oxygen supply is apparently not the limiting factor in these diver experiments. This view is further supported by the fact that in the course of the experiments the oxygen pressure within the diver does not decrease more than 2 mm. Hg at the most, whereas it is generally agreed (cf. Tang, 1941) that the sea-urchin egg respiration is unaffected by a decrease in oxygen pressure from 160 mm. down to 40 mm. The number of cells per volume of cell suspension is about the same in diver experiments as in Warburg ones, or slightly lower.

After completed diver measurements the cells were washed out of the divers with sea water, re-counted and then microscopically observed as to condition and development. In applicable cases even fertilizability controls were undertaken. Only those experiments were accepted in which the cells passed these post-diver-measurement controls.

2.3. *Evaluation of results*

The oxygen used up during the experiment, δv, is calculated from the read pressure difference, δp, according to the formula

$$\delta v = \frac{V \cdot \delta p \cdot T_0}{p_0 \cdot T}. \tag{1}$$

Using this formula δv will be given in µl. measured at 0° C. and normal barometric pressure, provided δp is stated in cm. Brodie, p_0 is the normal barometric pressure in cm. Brodie (= 1000), T is the temperature of the experiment in °K., T_0 stands for 273° K., and if V stands for the total gas space in µl. of the charged diver at equilibrium pressure. It will be noted that this formula is similar to that used by Holter (1943) p. 466 in cases where the solubility of the measured gas is low, and where formed CO_2 is absorbed away, but a temperature correction has been added in order to render a comparison between the measurements possible even if the latter have been taken at different temperatures (see, however, below). In the formula the absorption of oxygen in the liquid phases of the charge has been disregarded as it is a very small quantity.

From the diver equation given by Linderström-Lang (1943) p. 363 the following expression for V may be derived:

$$V = g_D\left[\frac{1}{\phi_M} - \frac{1}{\phi_{Gl}}\right] + V_W\left[\frac{\phi_W}{\phi_M} - 1\right] + V_{Oi}\left[\frac{\phi_{Oi}}{\phi_M} - 1\right] \quad (2)$$

where g_D = weight of the empty diver in mg.,

V_W = volume in µl. of aqueous charge (*i.e.* in the present case: cell suspension in bottom drop + hydroxide solution in neck seal II),

V_{Oi} = volume in µl. of paraffin oil in neck seal I,

ϕ_W, ϕ_{Oi}, ϕ_M and ϕ_{Gl} = densities of the aqueous charge, the paraffin oil, the medium and the diver glass.

The formula may be shortened to:

$$V = g_D \cdot A + B + C \quad (3)$$

where $g_D \cdot A$ may be defined as the total gas space in µl. of the uncharged diver at equilibrium pressure. This is a constant characteristic of the individual diver. For calculating A the graph in Figure 1a may be of help. For a given medium (in this investigation Holter's medium, $\phi = 1.325$; cf. Holter (1943) p. 412, has been used) A is solely a function of ϕ_{Gl}.

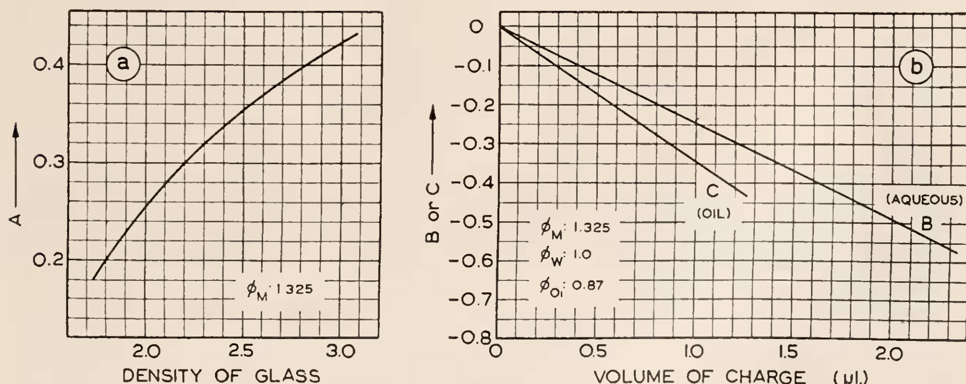

FIGURE 1. Graphs for calculating the values of the constants A, B and C in the formula for V, the total gas space of the charged diver at equilibrium pressure.

The constants B and C are governed by the adopted type of charge, but are independent of the characteristics of the diver. They may be found from the graphs in Figure 1b.

The value of A ought to be stated to the third figure; B and C will be sufficiently correct if stated to the second.

As stated in 2.2., measurements were usually performed at 18° C., but in some experiments on *Ps.* cells the temperature was varied within the limits 15–21° C. Measurements at different

temperatures are not comparable if merely calculated according to formula (1), as the cellular oxygen consumption rate is itself a function of temperature. As will be shown in a later paper (jointly with S. Lybing) this function in the unfertilized *Ps.* egg is characterized by a $Q_{10} \simeq 2.25$. Adopting this value, the consumption rates at any temperature within the range may be converted to consumption rates at 18° C. The same applies when the results of other investigators are compared with those given in this paper. The same Q_{10} value may, without significant errors, be used even for fertilized eggs.

If all corrections and constants are now put together the consumption formula (1) is simplified to:

$$\delta v = V \cdot \delta p \cdot K. \tag{4}$$

The value of K for different experimental temperatures when adopting $Q_{10} = 2.25$ will be found from Table I.

The technical errors of the method will no doubt remain below 5 per cent (cf. Holter, 1943). The biological scattering of the gained figures is, however, much greater. (This scattering is frequently met with by investigators who study oxygen consumption in marine invertebrates. Thus, using apparently identical objects one frequently comes across biological scattering amounting to the horrifying value of over 100 per cent.) In order to get a measure on the biological scattering in this investigation the standard deviation, σ, has been used. This

TABLE I

Value of K in the ultimate diver gas exchange formula if $Q_{10} = 2.25$

Temperature in experiment ° C.	K
14.5	1.264×10^{-3}
15	1.210
16	1.111
17	1.020
18	*0.938*
19	0.862
20	0.792
21	0.728
22	0.670
23	0.618

figure gives the limits within which about two-thirds of the experimental results will fall. In σ, of course, the technical errors of the method are incorporated. In addition, the significance of the given average values is stated by the standard error of the mean, ϵ, which gives the limits within which the real center of the biological (and technical) scattering is situated with a probability of about 70 per cent.

For *Par.* Lindahl and Holter (1940) state that there is a biological scattering in the egg volume of 10–15 per cent, in the enzyme content of the egg of about 10 per cent, and in the oxygen consumption (obtained by diver technique) of 10 per cent. In the respiration of *Ciona* eggs Holter and Zeuthen (1944) in diver experiments found a scattering of less than 20 per cent, and in the egg volume less than 16 per cent. (Cf. Zeuthen (1947a), who on pp. 44–48 has discussed the influence of biological factors on metabolic measurements, especially on such as are performed with diver technique.)

In the present investigation the standard deviations for respiration measurements are found in Tables II, III, V and VIII, and for egg size in Tables IV and VI. To summarize it may be stated that for *Ps.* respiration there has been found a relative σ slightly greater than 20 per cent, and for *Ast.* a σ slightly greater than 15 per cent. The scattering in size is markedly smaller: for cell diameter $\sigma = 4.3$ per cent in *Ps.* and 3.5 per cent in *Ast.*, and for cell volume 13.5 per cent and 10.9 per cent, respectively.

3. Experiments and Interpretations

3.1. *Respiration of oocytes and unfertilized eggs*

3.11. *Psammechinus*

3.111. *Cell material*

In *Ps.* the egg may still be unfertilizable though full nuclear maturity has been reached, *i.e.* the polar bodies are expelled. This unfertilizability is due to the well known fact that in sea-urchin eggs nuclear maturity is not followed by cytoplasmic maturity until considerably later. Such cytoplasmically immature *Ps.* eggs are characterized in the hypertonicity test (cf. Runnström and Monné, 1945) by rapidly appearing wrinkles which smooth out very slowly. These eggs are termed in this paper *under-ripe eggs.* When both nucleic and cytoplasmic maturity is reached, and the eggs can accordingly be fertilized, the wrinkles appear in 2–6 minutes after the eggs have been placed in hypertonic medium (2 ml. sea water + 0.6 ml. 2.5 N NaCl) and smooth out in about 40 minutes. Such totally mature eggs are here termed *ripe eggs.* Later on the eggs again cease to be fertilizable. The wrinkles of the hypertonicity test are now poor or fail to appear and, if any do appear, they smooth out rapidly. These eggs are termed *over-ripe* (cf. also Wicklund, 1947).

These three classes of egg maturity are not mixed in one and the same female but in a catch of animals, females belonging to all these classes may simultaneously be found. In the beginning of the spawning season the females with under-ripe eggs prevail. At the end of the season not only females with over-ripe eggs become frequent, but there is also a marked rise in the number of females with under-ripe eggs (tendency to second spawning period?). This corresponds to the fact that the number of oocytes in the extruded egg-mass is higher both at the beginning and at the end of the spawning season than at its height.

The size of the oocytes varies greatly. Only such as were fully grown were accepted for diver measurements. To facilitate this such egg material was preferred as showed low oocyte percentage, *i.e.* where the oocytes were already mainly transformed into eggs. Such a state seems to ensure full growth in most of the remaining oocytes. During the diver measurements some of the oocytes occasionally started meioses which, when the eggs were examined after the experiment, could be judged from the broken down nuclear membrane. Such circumstances, however, could never be proved to effect perceptibly the oxygen consumption figures. The number, if any, of developing oocytes was always low (cf. also Lindahl and Holter, 1941).

From the *Ps.* material it was accordingly possible to pick out four different kinds of cells for diver experiments, viz. oocytes, under-ripe eggs, ripe eggs and over-ripe eggs.

In some few cases cells deprived of their jelly hull (treatment with acid) were used because they acted as controls in other experiments on naked cells. According to Borei (1948) there is, however, very little difference, if any, in oxygen consumption between coated and naked cells.

3.112. *Respiration of ripe eggs*

3.112.1. *Respiration curve*

In the ovary the germinal cells are continuously supplied with fresh metabolic material and may thus keep their respiratory rate at a constant level. When shed they are cut off from any additional supply and their metabolic rate is bound to drop gradually. This view is supported by the experimental results; see Figure 2.

The earliest respiration values after removal from the ovary cannot be stated with the diver technique. The time required for washing and picking out the cells,

for filling the diver, plus a minimum of time for attaining temperature equilibrium and then for obtaining two measurements suitably apart, cannot be pressed down to much under 40 minutes. The earlier values may, however, be found by extrapolation, but the most interesting period, viz. the shedding, will only be described rather approximately by this method.

A gradual decrease in the oxygen consumption of the shed unfertilized egg has already been reported for the *Ciona* egg by Holter and Zeuthen (1944) using Cartesian diver technique. Lindahl and Holter (1941) in their diver experiments on *Par.* have not stated the time after removal from the ovary, but point out that it would probably have been of importance. In fact, the results presented in Figure 2 show how necessary such a precaution is if comparable values are desired.

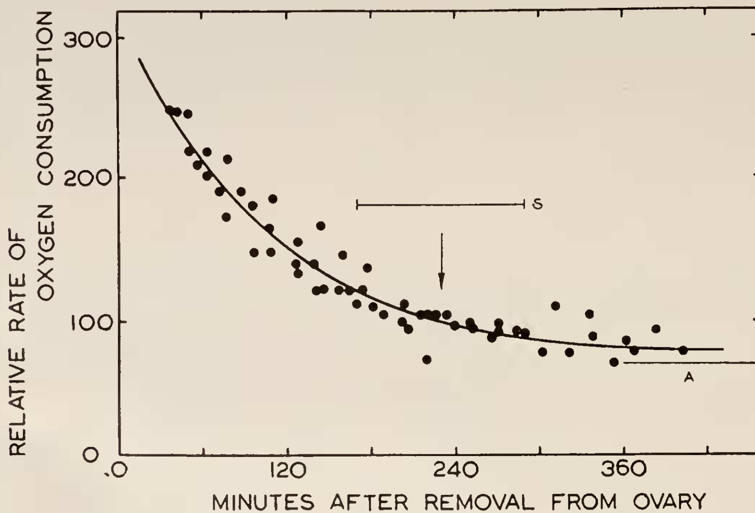

FIGURE 2. Relative rate of oxygen consumption of ripe *Psammechinus miliaris* eggs in relation to time after removal from ovary.

Results from 4 diver experiments. Values at 230 minutes (cf. 3.112.2) put = 100. Final asymptote (A) indicated. Arrow stands for chosen point of comparison for absolute rate measurements. Evaluated time span (S) in such experiments indicated.

The curve in Figure 2 closely resembles that of the endogeneous respiration of baker's yeast (Borei, 1942) where one is also concerned with cells cut off from external supply of metabolic material; the gradually decreasing respiration is composed of two parts. One proceeds monomolecularly and is thought to be governed by the rapidly decreasing amount of an initially present substrate. The other continues with a constant rate for a considerable time; its rate is thought to be limited by the available amount of an enzyme.

Mathematical analysis of the curve in Figure 2 shows that even the respiration of the unfertilized egg can be described in this way: There is a true monomolecular part of the respiration that rapidly tends to become zero, *i.e.* the curve approaches an asymptote (indicated in the figure). The amount below the asymptote represents the constant respiration part. This may probably be interpreted, as in the

case of yeast, as a simultaneous degradation of two different sources of metabolites over partly different enzyme systems.

3.112.2. *Absolute respiration values*

Because of the rapidly decreasing rate of oxygen consumption, as manifested in Figure 2, it is not convenient to compare values which are obtained from different materials during the first hours after the egg-removal from the ovary. Even small time differences may here cause considerable discrepancy in the consumption figure. Later on the curve becomes flatter and suitable for comparison with other experiments. The best thing to do would be to take the asymptote value, but this is impracticable by reason of experiment duration. Instead some point must be chosen on the part of the curve that has become reasonably horizontal, but which is still as close as possible to the point of egg-removal. Judging from the obtained curve, a point of time 4 hours from this event was chosen. In actual experiments 230 minutes from egg-removal was the average time, around which the measurements grouped themselves. Experimental conditions, however, have caused the center of the measurements in certain experiments to shift some 20 minutes in either direction. A total measurement-time of 2 hours, during which some six

TABLE II

Average rate (r) of oxygen consumption of ripe eggs of the two Psammechinus phenotypes 230 minutes after removal from ovary

n = number of diver experiments, each of which was performed on cell material from different females.

Phenotype	r (μl./egg/hour)	σ	ϵ	n	Difference in r (μl./egg/hour)
Z-form	0.47×10^{-4}	0.12	0.02	25	0.06×10^{-4}
S-form	0.53×10^{-4}	0.17	0.03	39	($\epsilon_{diff.} = 0.03$)

equilibrium readings could be obtained, was thought fit for the evaluation of the consumption figure. The average position on the curve of the final oxygen consumption figure and the time span of the measurements used for its evaluation are marked in Figure 2.

The results from measurements in this manner on ripe eggs of both the *Ps.* phenotypes are recorded in Table II.

The figures for the two phenotypes differ slightly. The difference is, however, not greater than twice its standard error. Thus it has not been possible to establish any significant difference in oxygen consumption rate between the ripe eggs of the two forms (concerning egg volume, cf. 3.114).

Borei (1934) studied the oxygen consumption of ripe *Ps.* eggs by means of Warburg technique (20° C.) and found, in accordance with the above results, the figure 0.52×10^{-4} μl. per egg and hour (converted to be comparable to the figures in Table II, whereby the egg volume value from Table IV has been used).

Using Barcroft differential manometers (14.5° C.) Shearer (1922b) got a considerably lower value for the ripe egg, viz. 0.20×10^{-4} μl./egg and hour (converted

to be comparable to the figures in the present investigation). This may be due to imperfect measuring technique (too tightly packed eggs).

3.113. *Respiration of oocytes, under-ripe eggs and over-ripe eggs*

To get oxygen consumption values comparable to those of the ripe eggs precautions were taken, similar to those mentioned above, concerning the measurements. It was found that even the oocytes showed a decreasing respiration similar to that of the ripe eggs. A special stickiness of the oocytes, as reported for *Par.* by Lindahl and Holter (1941), was not found in the *Ps.* material.

The oxygen consumption figures obtained for oocytes, under-ripe eggs and over-ripe eggs are given in Table III, where, for comparison, the average value for the ripe eggs (calculated from Table II) is also included. The number of experiments on the different cell types is, however, too small to permit of any comparison between the two *Ps.* phenotypes, as was possible in the case of the more abundant material on ripe eggs.

TABLE III

Average rate (r) of oxygen consumption of oocytes, under-ripe eggs and over-ripe eggs of Psammechinus 230 minutes after removal from ovary

n = number of diver experiments, each of which was performed on cell material from different females.

Cell type	r (µl./cell/hour)	σ	ϵ	n
Oocytes	0.66×10^{-4}	0.13	0.04	11
Under-ripe eggs	0.56×10^{-4}	0.17	0.06	10
Ripe eggs	0.51×10^{-4}	0.16	0.02	64
Over-ripe eggs	0.45×10^{-4}	0.09	0.04	7

Obviously there is a gradual, though slight, decrease in oxygen consumption on advancing cell maturation (all differences are statistically *well* established except that between under-ripe and ripe eggs, which is probable but not fully secured).

Although the findings on the *Ps.* material are thus not fully in accordance with those of Lindahl and Holter (1941) on *Par.* they, nevertheless, principally show a lower respiration in the ripe egg in comparison with that in the oocyte. This question will be discussed more extensively later on (see 4). On the other hand, the statement of Boell and co-workers (1940) that the ripe eggs of *Arbacia* consume more oxygen than the immature eggs (oocytes) contradicts these findings. (Possibly the oocytes used in their experiments had been kept for an especially long time in sea water before diver measurements began.)

The relatively low *Ps.* oocyte respiration in comparison with that of the *Par.* oocyte may possibly be connected with some sort of species-specific metabolic change caused by the contact with sea water when the cell is shed. In order to estimate the oocyte respiration inside the ovary an attempt was made to obtain cells immersed in ovarial fluid by means of introducing the tip of a braking micropipette through the ovary wall into the cavity. The habitus of the ovary, which made it very difficult to remove the coelomic fluid from the surface of the ovary, together with its great fragility, made it extremely difficult to draw out pure ovarial

content. Two additional factors invalidated the reliability of the diver measurements, viz. first, the dense packing of the cells inside the ovary, which tended to overcrowd the diver bottom drop thus giving bad gas exchange conditions and also causing great difficulties when the oocytes were to be picked out from the extracted material, and second, the frequent occurrence of other coelomic cells, such as amoebocytes, etc., which invariably and inseparably accompanied the desired cells. (Cf. similar experiments on *Ast.* oocytes in 3.122, where all these difficulties were mainly absent.) In any case the diver measurements indicated that the initial oocyte respiration in ovarial fluid is of the same order of magnitude as the initial one in sea water.

3.114. *Size of cells*

The two *Ps.* phenotypic varieties, the *Z*- and *S-forms,* were previously (3.112.2) compared as to the oxygen consumption of the ripe eggs. No significant difference could be established. An attempt was then made to correlate the great biological variability of the oxygen consumption to the size of the eggs. No correlation could, however, be established between these two entities. If the amount of protoplasmic material has any influence on the rate of oxygen consumption, as might have *a priori* been expected, this influence is obscured by other experimentally uncontrolled factors governing the metabolic rate and the size.

TABLE IV

Diameter (d) and volume (v) of ripe eggs from the two Psammechinus phenotypes

n = number of females examined. At least 20 eggs from every female were measured

Phenotype	d (μ)	σ	ϵ	n	Difference in d (μ)	v (μl.)
Z-form	94.6	3.5	1.1	11	9.1	4.43×10^{-4}
S-form	103.7	5.1	0.7	49	($\epsilon_{diff.} = 1.3$)	5.84×10^{-4}

On the other hand distinct differences in size between the ripe eggs of the two forms definitely exist, as can be seen from Table IV. The significance of the difference is established by the fact that it is 7 times as big as its standard error. Similar results have been gained by Lindahl and Runnström (1929) (*Z*-eggs $d = 98.3\,\mu$; *S*-eggs $d = 114.8\,\mu$).

The size of the eggs is not a given constant. Thus, for instance, the *Z-form* eggs were found to be smaller in 1946 than in 1947 ($d = 87.5\,\mu$ and $d = 94.6\,\mu$, respectively) and Lindahl and Runnström (1929) state $d = 98.3\,\mu$ for eggs of animals from the same district in the late Twenties. Moreover, geographical dissimilarities also seem to exist: Thus Laser and Rothschild (1939) give $d = 103.3\,\mu$ for a Millport material and Hobson (1932) gives a volume corresponding to $d = 118.4\,\mu$ for a Plymouth material. These two materials are no doubt of *Z-form,* for which the figures $d = 87.5$–$98.3\,\mu$ were found at the Swedish West Coast (see above). Hydrographically caused nutritional differences are probably the chief reason for these dissimilarities.

Because of the simultaneous existence of full size oocytes and a variety of small ones, which are not fully mature, it is rather difficult to get a reliable figure for the

oocyte diameter. However, the oocytes seem, on an average, to be smaller than the eggs. This is supported by all measurements on material with apparently homogeneous oocytes, especially in the cases in which the oocyte percentage is low. For example a material with an average egg diameter of 96.8 μ showed an average oocyte diameter of 88.8 μ. These findings are in concordance with the results on *Ast.* (see 3.123), where the oocytes are quite homogeneous in size and where the eggs are bigger than the oocytes. Similar results are reported by Lindahl and Holter (1941) in *Par.*

It may be noted, first, that in *Ast.* the (primary) oocytes respire at a much lower rate than the eggs (or secondary oocytes), second, that in *Ps.* the oocyte respiration is instead slightly higher than the egg respiration, and third, according to Lindahl and Holter (1941), that in *Par.* the oocytes respire at a much higher rate than do the eggs. Thus the respiration cannot be correlated to the cell size and furthermore, as the amount of protoplasm will always be nearly the same in oocytes and eggs (though the degree of hydration may differ), it naturally cannot be correlated to the content of organic matter.

3.12. *Asterias*

3.121. *Cell material*

For *Asterias forbesii* Costello (1935) states that the nuclear membrane of the oocytes begins to disappear after about 10 minutes in sea water (19–26° C.) and that the first polar body is separated in about 60 minutes. After another 20 minutes the second polar body appears. Cytoplasmic maturity seems to be attained rather rapidly and to be connected with the diffusion of the nuclear sap into the cytoplasm. Chambers and Chambers (1940) confirm the maturation time schedule.

This scheme obviously also holds for the *Ast.* material of this investigation. When taken from the ovary the cells are, without exception, oocytes. (Recent authors agree that on natural spawning the cells are in a state of broken down germinal vesicle, *i.e.* primary oocytes with initiated first meiosis; cf. Hörstadius, 1939, and Runnström, 1944.) Within 30–40 minutes after being placed in sea water (18° C.) they usually show broken down nuclear membranes in over 90 per cent of the cells. At 60 minutes the first polar body can usually be seen and at 120 minutes often two (or three) are formed. The polar bodies are frequently dissimilar in size.

The natural stimulus for the oocyte development is the sea water (cf., *e.g.*, Hörstadius, 1939). This is clearly shown by the experiments with oocytes in ovarial fluid (3.122), where the meiosis was delayed for several hours. Small contaminations with sea water (or coelomic fluid), however, caused the seminal vesicle to break down rapidly.

After a few hours in sea water the cell material mainly consisted of eggs and a certain small percentage of primary oocytes. In addition, there were a number of secondary oocytes. These, however, were rather hard to distinguish from the eggs because they look exactly the same as the eggs. The only difference lies in the number of polar bodies, and this is extremely difficult to determine when picking out the cells with a braking pipette. Thus the material referred to as "eggs" in the diver experiments, may consist of a mixture of secondary oocytes and eggs. However, the respiration of these two kinds of cells seems to differ only very slightly, since the "egg" respiration agrees very well with that of the young fertilized egg (cf. 3.122). When taken out of the diver after the measurements and then more closely examined under the microscope most of the cells proved to be true eggs. Nevertheless the second meiosis might, of course, have taken place inside the diver during the oxygen consumption measurements.

Any noticeable break in respiration rate could, however, never be observed during diver measurements. Usually the "eggs" were picked out for the diver charge during the second hour in sea water.

The statement of Loeb and Wasteneys (1912) on unfertilized *Asterias forbesii* eggs, that these rapidly die if their oxidative processes are not suppressed, may also be true for the present material. In fact after some 24 hours a "dark" cytolysis is very common. For the diver measurements, which were mostly concluded within 4 hours after the removal from the ovary, this obstacle seemed to be without significance.

For the diver measurements on primary oocytes, such ones as have been mentioned before and which pass quickly over to eggs could not be used. Being a very homogeneous material they would have been very suitable, but measurements could hardly have started before they were already transformed. Thus for the diver experiments only such oocytes as remain as oocytes after some hours in sea water could be utilized. These cells have exactly the same appearance and size as the rapidly converting oocytes. The fact that they remain unconverted seems to point to some sort of immaturity. This might be the case, but only to a certain extent, for the majority of these eggs do actually convert later on, *i.e.* within some few hours after they have been taken out of the diver again for purposes of control. Thus one might be justified in thinking that the figures obtained on this material would not differ very much from those on rapidly converting oocytes, could they have been obtained. This view is supported by the fact that the obtained consumption figures are the same, irrespective of when the future conversion takes place and by the fact that it is possible to correct the obtained values for the oxygen consumption of such oocytes as have their germinal vesicle broken down when inside the diver (by applying the consumption value for eggs). Moreover, experiments with oocytes in ovarial fluid (in this case mostly "rapid" oocytes must be present) show consumption figures of the same order of magnitude as oocytes in sea water but not as eggs in sea water.

Only such oocyte experiments were accepted as, when examined immediately after the completion of the diver measurements, show no or very few oocytes with broken down germinal vesicle. In the latter case a correction could be applied, if the number of converted oocytes was low.

Control experiments on fertilizability were always performed. They proved that the fertilization percentage was always remarkably low, rarely over 50 per cent and often around 20 per cent. This may partly be due to a lowered mobility on the part of the sperms (reason unknown) and partly to improper sea water conditions (see 2.2). Earlier investigators have stated that 100 per cent fertilization can only occur in connection with natural spawning, artificial fertilization always giving a poor yield.

It may be noted that depriving the *Ast.* cells of their jelly hull has as negligible an effect on the oxygen consumption figure as has the same procedure on *Ps.* cells. Thus an experiment with three parallel divers on naked and on coated *Ast.* eggs from the same female gave the average consumption figures of 2.31 and 2.39×10^{-4} μl. per egg and hour, respectively.

3.122. *Respiration of oocytes and eggs*

Even in *Ast.* cells there is a decreasing oxygen consumption rate as has already been described for ripe *Ps.* eggs (cf. 3.112.1). The rate of decrease is not so steep, however, as in *Ps.* Thus to obtain comparable figures, it ought to be possible to use measurements nearer the point of removal from the ovary than in *Ps.* Nevertheless, the transformation of oocytes into eggs described in the preceding chapter makes early measurements impossible. In the second hour after removal this transformation is practically completed. It is thus most convenient to start evaluating measurements about 120 minutes after removal. The time span of the evaluated measurements has, in the case of the *Ast.* experiments also been about 2 hours. This puts the average point of time used for comparison between different experiments (cf. 3.112.2) to 180 minutes after cell removal from the ovary. The same point of time has been used for both oocytes and eggs.

In order to investigate the cell respiration inside the ovary, experiments on

cells in ovarial fluid were carried out in complete accordance with those on *Ps.* material (cf. 3.113). The difficulties met with in *Ps.* were not at all present in *Ast.* Firstly, the ovarial cells are, without exception, oocytes which make any secondary selection unnecessary. Secondly, the *Ast.* cells are not so densely packed in the ovary, and amoebocytes, etc., are far fewer. Moreover, the form and comparative strength of the ovary makes it easier to free it from sea water or coelomic fluid on the outside. These experiments were started, however, so late in the spawning season that but few can be recorded here. They merely show a general oxygen consumption similar to that of oocytes in sea water.

The results on oocytes and eggs in sea water and on oocytes in ovarial fluid are given in Table V.

Perhaps it is justifiable to think that the results reported in this chapter indicate that the oocytes inside the ovary have a relatively high oxygen consumption velocity owing to the external supply of metabolically utilizable substrate from the ovary.

TABLE V

Average rate (r) *of oxygen consumption of oocytes and eggs of Asterias 180 minutes after removal from ovary*

n = number of diver experiments, each of which was performed on cell material from different females.

Cell type	r (μl./cell/hour)	σ	ϵ	n
Eggs in sea water	2.50×10^{-4}	0.33	0.08	18
Oocytes in sea water	1.15×10^{-4}	0.28	0.09	10
Oocytes in ovarial fluid	1.36×10^{-4}	—	—	2

When shed in sea water (or taken out of the ovary) and thus cut off from this supply the rate gradually drops. On transformation into secondary oocytes the rate is considerably raised, but even in this state there is a gradually diminishing oxygen consumption rate.

The difference between eggs and oocytes in sea water is unquestionable (difference = 1.35; $\epsilon_{diff.} = 0.12$). Tang (1931) using Warburg technique (23° C.) arrived at a considerably lower respiration figure for *Asterias* eggs, viz. 1.14×10^{-4} μl./egg and hour (converted to be comparable to the figure in the present investigation), than that obtained in this investigation. Possibly Tang used another species (species not stated; *forbesii* (?), Woods Hole). Tang's value of the size of the egg ($d = 160\,\mu$) does not, however, differ appreciably from that of *Ast.* (cf. 3.123). It might therefore be natural to conclude that the two consumption values ought to have agreed. The rapid shaking rate of the Warburg vessels in Tang's experiments might probably have caused damage to the eggs. Moreover, Tang could not find any difference in the rate of respiration between eggs and oocytes. Boell and co-workers (1940) using diver technique were also unable to state any difference between eggs and oocytes. The close similarity between their experimental conditions and those of the present investigation makes their results quite inexplicable. (Boell and co-workers have hitherto only published their results as a preliminary note.) On the other hand Brooks (1943) in Warburg

experiments on *Asterias forbesii* finds a lower oxygen consumption in oocytes than in eggs.

3.123. *Size of cells*

The size of the *Ast.* egg is given in Table VI. Just as in *Ps.* (cf. 3.114) and in *Par.* (Lindahl and Holter, 1941) the oocytes are slightly smaller than the eggs, as can be seen from Table VII. This difference in volume is, however, definitely too small to account for the found difference in oxygen consumption.

The eggs from the same ovary are often more constant in size in *Ast.* than in *Ps.* The average relative difference (calculated on eggs from 10 females) from the mean diameter were 2.9 per cent ($n = 200$) for *Ast.* and 3.6 per cent ($n = 200$) for *Ps.* eggs.

TABLE VI

Diameter (d) and volume (v) of Asterias eggs
n = number of females examined.
At least 20 eggs from every female were measured.

d (μ)	σ	ϵ	n	v (μl.)
169.1	5.9	1.4	18	25.2×10^{-4}

TABLE VII

Comparison between cell diameters of Asterias oocytes and eggs
Each figure represents the mean of 20 measurements

Female No.	Oocytes	Eggs	Eggs/Oocytes
1	149.9μ	157.4μ	1.05
2	151.9	157.8	1.04
3	176.0	180.4	1.03
4	156.6	163.1	1.04
5	165.6	169.9	1.03

3.2. *Respiration before and after fertilization*

3.21. *Psammechinus*

In order to follow the oxygen consumption changes induced by activation an attempt was made to perform the fertilization inside the diver during actual measurements. A diver charge type with a diver neck wall drop containing the spermatozoa in sea water was employed (for details cf. "Diver charge Type II" in Borei, 1948). The spermatozoa, however, always crept over the dividing glass surface and gradually reached the eggs before the neck wall drop and the neck seal II were purposely brought together. This premature fertilization was apparently facilitated by the condensed moisture that always prevails on the inside glass surface of the diver. To measure the respiration of the young fertilized egg one is thus obliged to use cells that are fertilized outside the diver. In this case one must be satisfied if the first point of measurement can be obtained at about 40 minutes after fertilization, because charging the diver etc. takes some time.

After the instant rise in oxygen consumption after fertilization, an exponentially increasing part of the respiration curve (Gray, 1926) starts. For the first hours this increase is still quite moderate, thus leaving the curve rather flat. During this time the curve is especially suitable for obtaining comparative values from different egg material. In the actual experiments of this investigation the first evaluated measurement was chosen to be about 60 minutes after fertilization and the last at 180 minutes, thus fixing the point of time for comparison between the different experiments to about 2 hours after fertilization. The results are given in Table VIII.

TABLE VIII

Average rate (r) of oxygen consumption of fertilized eggs of Psammechinus 120 minutes after fertilization

n = number of diver experiments, each of which was performed on cell material from different females.

r (µl./embryo/hour)	σ	ϵ	n
1.84×10^{-4}	0.31	0.06	30

No difference between the two *Ps.* phenotypes could be found (*Ps. Z-form* 1.81×10^{-4} µl., $n = 9$; *Ps. S-form* 1.85×10^{-4} µl., $n = 21$).

Comparing the respiration value of the fertilized egg (Table VIII) with that of the ripe unfertilized egg (Table II) one finds a ratio of 3.6. With *Ps.* eggs Shearer (1922b) using a Barcroft differential manometer technique (14.5° C.) previously found a ratio of 5.7. His measurements on fertilized eggs fall within 30–60 minutes after fertilization. In the case of the unfertilized eggs one cannot judge how long a time elapsed between the removal from the ovary until the measurements were made. This point is certainly of great importance. The earlier the measurements are made the higher the consumption rate of the unfertilized egg will be (cf. Fig. 2). And consequently the higher the rate of the unfertilized egg the smaller the ratio between the oxygen consumptions of the fertilized and unfertilized egg will probably be. In this connection it might be mentioned that the influence of the respiration of the unfertilized egg (at the moment of fertilization) on the future respiration of the fertilized egg is unknown. It is quite possible that a high respiration of the unfertilized egg at the point of fertilization, *i.e.* a still high content of substrate utilizable in the unfertilized state, may be reflected in a relatively higher respiration of the fertilized egg, at least during the first hours.

The value of Borei (1934) on fertilized eggs cannot be used for comparison in this connection because it was referred to the volume of the egg including the fertilization membrane. Owing to the very variable size of this membrane the actual number of eggs in these experiments cannot be calculated.

On material from other sea-urchin species a variety of ratios have been obtained. Only a few will be quoted here. They are mainly taken from the investigations and reviews of Whitaker (1933), Ballentine (1940) and Lindahl and Holter (1941):

Arbacia punctulata	2.6, 3.9, 4.4, 4.5 5.0, 5.0, 5.0, 5.3
Arbacia pustolosa	6.0
Psammechinus microtuberculatus	3.8
Paracentrotus lividus	3.1, 6.0

The cited material is very heterogeneous, representing very different techniques of measurement (such as Winkler titration, Warburg measurements and Cartesian diver experiments) and very differing temperature conditions (15–26° C.). Moreover the actual time after removal from the ovary is never stated for the unfertilized eggs (the most reasonable assumption being that a few hours have elapsed) and in the case of the fertilized eggs the time after fertilization is sometimes uncertain or completely omitted. Thus the significance of the figures is plainly qualitative. They only show that the oxygen consumption of the unfertilized eggs some few hours after their removal from the ovary is considerably lower than that of the fertilized eggs during the first hours after fertilization.

Gray (1926) found that in *Ps.* the rise of oxygen consumption after fertilization followed an exponential curve. Lindahl and Öhman (1938) working on *Par.* were able to confirm this, and Lindahl (1939) established the S-shaped curve characteristic for the oxygen consumption until hatching. After this there is a sudden steep rise associated with the onset of further growth and morphological modifications.

This shape of the respiration curve after fertilization has been perfectly confirmed in this investigation by diver experiments, which are presented in Figure 3. For comparison the curves of Gray, Lindahl and Borei have been plotted on a relative scale (putting the values 30 minutes after fertilization alike) in the small right hand bottom graph. To complete the survey the curve of the correspondingly run diver with unfertilized eggs has also been plotted in the main graph. In this the times of the first mitoses (judged from microscopically observed parallel cultures) and those of ciliation, hatching etc. have also been indicated. It must be observed that the time which elapses before the hatching of the embryos is somewhat longer inside the diver than in parallel ordinary cultures, viz. 9 and 8.5 hours, respectively (cf. 3.3). This delay is always observed and must be ascribed to the relatively dense packing inside the diver. If the embryos, after hatching, are washed out of the diver and cultured in the ordinary way they develop into perfectly normal plutei.

Zeuthen (1947b) working with refined diver technique on *Ps.* (16° C.) was able to show that the primary exponential oxygen consumption curve has waves superimposed upon it. The minima of these waves correspond to the first cleavages (telophase) of the egg. His main graph must, however, be incorrect as to the time position of the cleavages, *e.g.* in the main graph the fifth mitoses is indicated at 5 hours after fertilization, whereas it correctly occurs at about 3 hours and 20 minutes. Assuming, however, that in his small graph the time mark between the fourth and fifth mitoses stands for 3 hours after fertilization the values are in concordance with known cleavage rates. (In Figure 3 of the present paper the first cleavages are marked in their right position on the curve). Moreover, in order to get an entire graphical scheme of the oxygen consumption of the reproduction cell of the female sea-urchin, Zeuthen has completed his *Ps.* curve with the results of Lindahl and Holter (1941) on oocytes and unfertilized eggs of *Par.*, and with

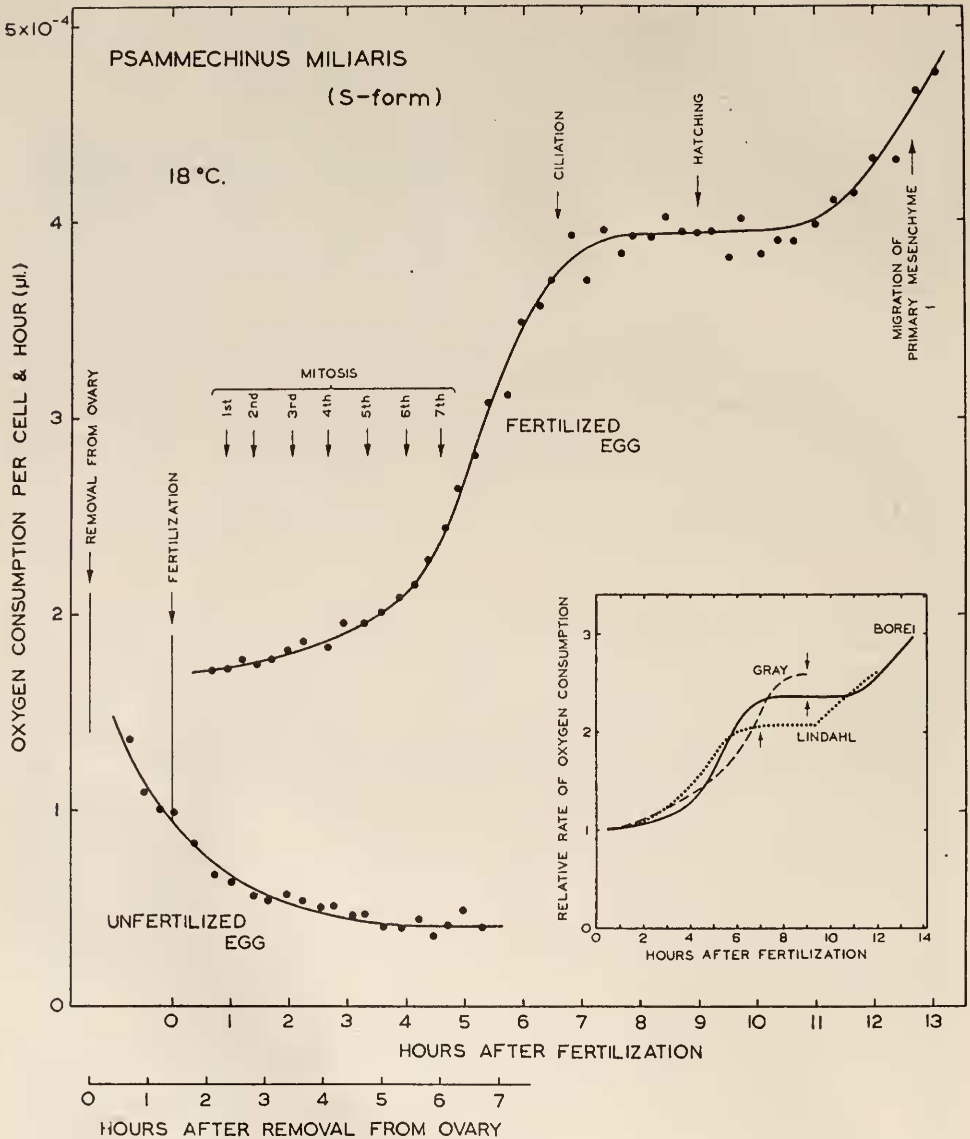

FIGURE 3. Oxygen consumption of *Psammechinus* eggs before and after fertilization.
The values are obtained from parallel divers, one with unfertilized, the other with fertilized eggs. For comparison the results of Gray (1926) on *Psammechinus miliaris* (17° C.) and of Lindahl (1939) on *Paracentrotus lividus* (22° C.) together with those of this investigation (18° C.) are plotted on a relative scale (extrapolated values at 30 minutes after fertilization = 100) in the small right hand bottom graph. In this graph the times of hatching are indicated by arrows. In the main graph the position of the telophase (appearance of cleavage furrow) of the earlier mitoses is indicated.

the results of Lindahl (1939) on fertilized eggs of *Par.* From the results on *Ps.* oocytes and unfertilized *Ps.* eggs presented in this paper (cf. 3.112 and 3.113) it is apparent that the respiration of the *Ps.* and *Par.* cells differs. Thus results from the two species must not be represented as a continuation in one and the same curve.

As has been previously mentioned it has not been possible to use the diver technique to measure the respiration until 40 minutes after the fertilization. Before this time there is said, however, to be a higher oxygen consumption than at any time during the next few hours. This was first indicated in measurements of Shearer (1922a) on *Psammechinus microtuberculatus* and later studied in detail by Runnström (1933) and by Laser and Rothschild (1939) working on *Par.* and *Ps.*, respectively. Nevertheless the exact shape of this part of the curve is still uncertain. (The slight temporary rise over the unfertilized egg value immediately after fertilization, which is indicated in Zeuthen (1947b) in his main graph, is not drawn in accordance with the findings of the mentioned investigators.) The period between fertilization and the first diver measurements has been left empty in Figure 3, but one must, however, keep in mind that during this period oxygen consumption rates, higher than that at 40 minutes, may have occurred.

3.22. *Asterias*

Comparing the respiration rates of unfertilized and fertilized *Asterias* eggs Loeb and Wasteneys (1912) (Winkler measurements) and Tang (1931) (Warburg technique) found no differences. In Cartesian diver experiments Boell and co-workers (1940) confirmed this. The results in the present paper in no way differ from these results. Here the respiration has been followed, however, for a longer space of time than in the earlier investigations, namely over a period of more than 200 minutes after fertilization, *i.e.* over the first mitosis. A gradual increase in oxygen consumption rate is to be noted during this time, as will be seen from Figure 4 (concerning the cell material, cf. 3.121).

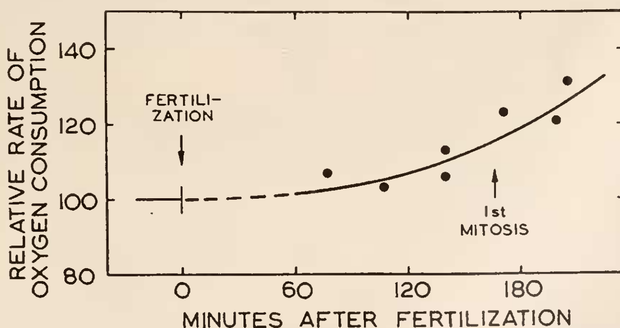

FIGURE 4. Oxygen consumption of *Asterias* eggs before and after fertilization.
The exact position of the first mitosis telophase in relation to the time of fertilization depends on the state of the egg when fertilized (secondary oocyte or egg). In a batch of cells mitosis may thus not occur synchronously.

3.3. *Cleavage rate*

In previous chapters reference has been made to the succession of mitoses and the time of their occurrence after fertilization. Though this cleavage rate in *Ps.* has repeatedly been studied by various authors, it has, for the sake of control, been checked even in this investiga-

Table IX

Cleavage rate of fertilized Psammechinus eggs

Concerning right time values in Zeuthen's experiments cf. 3.21

Author	Gray (1926)	Zeuthen (1947b)	Borei (this investig.)
Temperature	17° C.	16° C.	18° C.
Salinity	34–35‰	32‰	33.2‰
	Time after fertilization:		
1st mitosis	67 mins.	—	56 mins.
2nd	100	—	83
3rd	132	—	127
4th	168	165 mins.	160
5th	203	200	~200
6th	238	240	~240
7th	271	(290)	—
Hatching	9 hours	9 hours 30 mins.	8 hours 35 mins.

tion. Found discrepancies were, however, with exception of the first cleavage time, of minor importance as is shown in Table IX.

It should be noted that in this table the values of Borei and Gray were obtained from cultures, whereas Zeuthen's are from observations on dividing eggs inside a diver. In this latter case the hatching time is somewhat delayed. As has been mentioned before (3.21) even in the present investigation a minor delay has been observed in cells when inside a diver.

In Figure 5 the cleavage sequence for *Ps.* and *Ast.* has been represented graphically. For comparison the cleavage rate for the irregular sea-urchin *Echinocardium cordatum* has been plotted in the same figure. All values were obtained during this investigation.

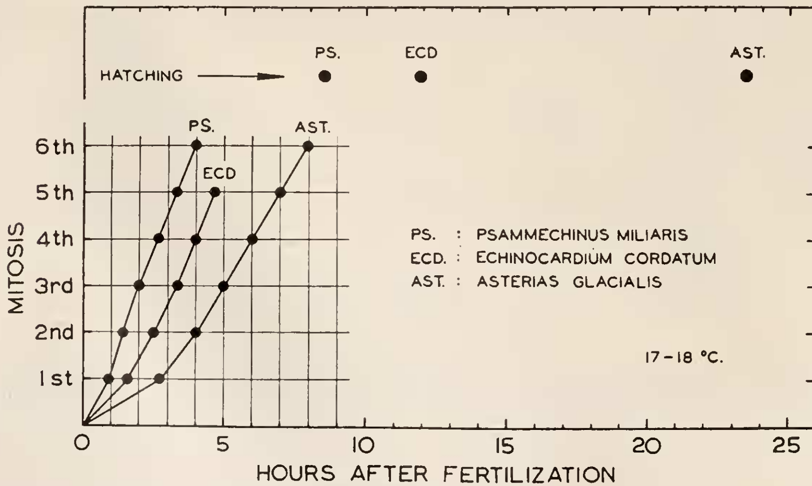

Figure 5. Cleavage rate in *Psammechinus, Asterias* and *Echinocardium.*

Values obtained from observations on sparse cultures (200 eggs in 10 ml. sea water). The mitoses are represented from the appearance of the cleavage furrow (teleophase). The position of the first cleavage in *Ast.* is dependent on the state of the egg when fertilized; in the represented sequence the germinal vesicle had just broken down when the sperms were added.

4. General Discussion

Many investigators consider that they have reason to look upon the mature sea-urchin egg as a resting cell between the growing oocyte and the developing fertilized egg. (For literature see Lindahl and Holter, 1941.) The oxygen consumption measurements with diver technique performed by Lindahl and Holter (1941) on *Par.* oocytes, mature unfertilized eggs and fertilized eggs seem to give excellent support to this view. They found that the primary oocytes apparently have a respiration even slightly higher than that of the newly fertilized egg, whereas the unfertilized eggs have a considerably lower oxygen consumption. The authors compare the state of the unfertilized egg with the diapause of certain insect eggs. The investigations of Whitaker (1933) indicate that the low unfertilized egg respiration in comparison with that of the fertilized egg is, among marine invertebrates, peculiar to the sea-urchin group.

The experiments in this paper show, however, that the sea-urchin group is not quite homogeneous in respect to oocyte respiration, as may be seen from Figure 6. Though the oocyte oxygen consumption was measured in principally the same way as in Lindahl and Holter's investigation it was found that *Ps.* oocytes, in contradistinction to those of *Par.,* consumed oxygen at a rather low rate, not differing very much from that of the unfertilized egg.

A new fact, however, is revealed in this investigation concerning the sea-urchin egg respiration, viz. the rapidly decreasing rate of oxygen consumption after the cell has been removed from the ovary (cf. Fig. 2). This declining respiration rapidly and asymptotically approaches a considerably lower value than the initial one (cf. parallels in the endogenous respiration of baker's yeast, kinetically studied by Borei, 1942, and briefly reviewed in 3.112.1). According to these facts the initial rate of oxygen consumption upon egg removal may even be higher than the rate of the fertilized egg during the first hours after activation (cf. Fig. 3). Such a rapidly declining respiration has also been found to be characteristic of the *Ps.* oocytes (3.113).

In all previous investigations the cell material must undoubtedly, though never stated, have been at least some hours old (reckoned from the time of removal from the ovary) when used in consumption measurements. Thus in the present investigation only such *Ps.* and *Ast.* measurements as were obtained from material of that age may be compared with the values of the previous authors. This means that only the measurements on the low level stage which are given in 3.112.2, 3.113 and 3.122 and summarized in Figure 6 may be used for comparison.

If differences now exist, as they probably do, both between the species and between the different kinds of cells within the species, as regards the rapidity with which the low level state is reached, *i.e.,* in steepness of the declining curve, it might in the *Par.* case be that one has hit upon oocytes which show a very slow decrease, whereas in the *Ps.* case the oocytes show a rapid decrease. Under such circumstances the apparent contradictions between *Ps.* and *Par.* oocyte respiration can be understood.

In the case of Boell and co-workers' (1940) findings in *Arbacia* of a lower respiration in the oocytes than in the eggs, it might be either that the oocyte respiration decrease is especially rapid or that the respiration decrease of the eggs is especially slow in that species, or it might be that the time which has elapsed since the removal from the ovary is longer for the oocytes. As no technical points

whatsoever are stated in Boell and co-workers' paper it is impossible to draw any conclusions.

It is very interesting to discover that there is general agreement within the sea-urchin group as to a low respiration in the unfertilized egg (when measured some hours after removal from the ovary) in comparison with the respiration of the fertilized cell. (The reviews of Whitaker (1933) and Ballantine (1940) and the results given in 3.21 might be consulted.) Now Loeb and Wasteneys (1912) have found that unfertilized *Asterias* eggs which respire at the same rate as the

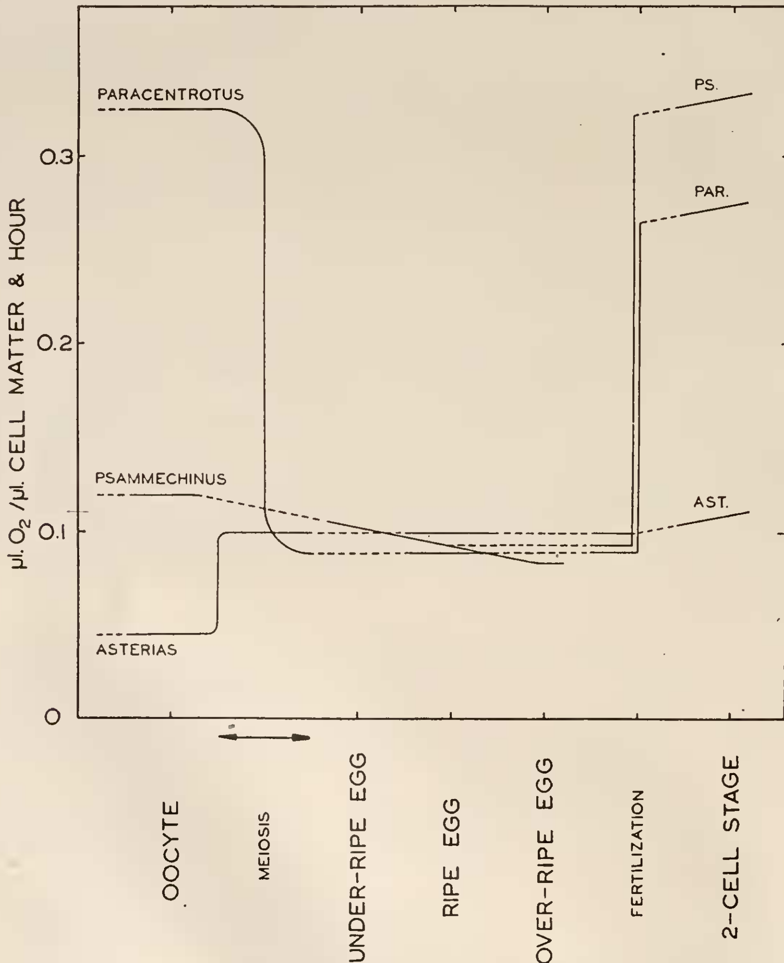

FIGURE 6. Comparison on a cell volume basis between the oxygen consumption of different female reproduction cells from *Psammechinus, Paracentrotus* and *Asterias*.

The results on *Ps.* and *Ast.* are from the present investigation; those on *Par.* from Lindahl and Holter's (1941). In the unfertilized cells the oxygen consumption was always measured several hours after cell removal from the ovary. All rates are uniformly calculated for 18° C. and expressed as if measured at 0° C. and at normal barometric pressure. Average cell volume values used: *Ps.* 5.56×10^{-4} μl., *Par.* 5.75×10^{-4} μl. and *Ast.* 25.2×10^{-4} μl.

fertilized ones, die very rapidly if not kept anaerobically (*i.e.*, if the oxidative processes within the eggs are allowed to continue at an unlimited high level). In view of this and the considerations of other authors one is inclined to look upon the low respiration of the sea-urchin egg as a natural precaution in order to facilitate long life for the shed egg. In this connection one must consider the actual conditions of the localities at time of spawning. The animals there live rather close together and spawning probably sweeps epidemically and simultaneously over the specimens, both male and female, of a given species in a community. Thus a rapid fertilization may be secured, which would mean that the egg respiration would only have time to enter the very first part of the declining curve. It is possible that eggs, which accidentally are not fertilized at once, are preserved for future activation through this low-level mechanism, which thus promotes the reproduction possibilities of the group. On the other hand, however, the similar declining curve of the oocyte respiration will probably have no biological significance whatsoever because, as is generally agreed, the sea-urchin oocytes are never shed.

The biochemical mechanism that causes the differences in respiration between fertilized and unfertilized sea-urchin eggs, as studied by Runnström (1930, 1933, 1935), Örström (1932), Korr (1937) and others, is likely to be characterized in the unfertilized egg by a block in the chain of carriers which, in the fertilized egg, mediates the oxidation of the substrates. This chain of carriers is supposed to include the cytochrome-system. Runnström considered that the oxidase was unsaturated with its substrate, and later investigations have furthered the view that substrates, dehydrogenases and oxidase are kept apart in the unfertilized egg. It is remarkable that the respiration of the unfertilized egg is insensitive to inhibitors affecting the cytochrome-system, whereas the respiration of the fertilized egg is very sensitive. In all these investigations it has been pointed out that the respiration of the unfertilized egg is low in comparison with that of the fertilized egg. This means that the studies have been performed on unfertilized eggs, in which the declining respiration part has already reached a low level. As the initial respiration of the unfertilized egg (when just removed from the ovary) can be even higher than that of the fertilized egg, the question therefore arises as to the relation between the rapidly declining (monomolecular) respiration part of the unfertilized egg and the respiration of the fertilized egg. Whether or not the oxidase is as important for this part of the respiration of the unfertilized egg as it is for the fertilized egg can probably be settled if inhibition experiments are performed as early as possible after the eggs have been removed from the ovary, *i.e.* when the declining respiration is still prominent.

The oxygen consumption figures in the three stages of ripeness of the unfertilized *Ps.* egg reveal that the respiration value slightly decreases with growing ripeness (cf. 3.113 and Fig. 6). It has not been possible to establish any difference in the early declining respiration of these stages. The material is, however, too small for convincing interpretations. (It should be kept in mind that the respiration of unfertilized sea-urchin eggs when kept for a long time in sea water undergoes a change, thus becoming more like that of the fertilized egg.)

In the case of the *Ast.* egg it has been found that the respiration is lower in the primary oocyte than in either the secondary oocyte or the unfertilized egg (cf. 3.122 and Fig. 6), but this finding is in contradiction to earlier ones (Tang, 1931, and Boell and co-workers, 1940; cf., however, Brooks, 1943). As the eggs on natural

spawning are shed with broken down germinal vesicle, *i.e.* as secondary oocytes or as eggs (cf. Runnström, 1944), the jump in respiration rate between primary and secondary oocytes does not occur outside the ovary. The respiration after fertilization proceeds (as has been reported by several previous investigators) at much the same level as before. As the decreasing part of the respiration of the cells after their removal from the ovary is not very marked in this material, the rate of respiration of the fertilized eggs may not, under natural conditions, differ very much from that found in this investigation (3.22), even if the fertilization takes place very soon after spawning. Thus if the rates of respiration of the fertilized sea-urchin eggs and the fertilized starfish eggs are compared on a cell volume basis (see Fig. 6), a very much lower respiration in the case of the starfish will be found. Whitaker (1933) has argued that the oxygen consumption of fertilized marine animal eggs from several invertebrate phyla and even of other developing cells, when compared on a cell volume basis, will show a remarkable consistency, whereas the respiration values of the unfertilized cells are widely scattered. It may be read from Whitaker's discussion, though not stated in these terms, that the respiration of the fertilized eggs is thought to be intimately connected with the work of morphogenesis and with biochemical activities connected with growth, and that in developing cells the amount of oxygen required for these purposes is about equal per volume of cytoplasmic matter. It is stated, however, that big cells, especially yolky eggs respire at a much lower rate. Other factors to be considered will, no doubt, be different degrees of cytoplasmic hydration, inert inclusions in vacuoles, etc., dead protecting or otherwise supporting structures, etc. The measurements by Tang (1931) on *Ast.* egg respiration before and after fertilization gave too low values to fit in Whitaker's scheme. The latter author severely criticizes technical weaknesses in Tang's measurements and leaves them out of his survey. The present investigation has certainly found Tang's values to be notably low (cf. 3.122), but still the fertilized *Ast.* egg respiration is remarkably low in comparison with that of the sea-urchin egg (cf. Fig. 6). It should be borne in mind that the *Ast.* egg has a volume that is about 5 times greater than that of the *Ps.* or *Par.* egg. This fact may probably, in the light of the discussion on the connection between body size and metabolic rate recently put forward by Zeuthen (1947a), be of more importance than considerations concerning supposed morphogenetic work.

To summarize the egg respiratory conditions found in this investigation together with those previously known, the graphical schemes in Figure 7 may serve.

The *Ps.* ripe egg (Fig. 7a) (cf. 3.112.1) has very probably a high respiration level in the ovary; at least it starts with high respiration velocity when brought into sea water. The oxygen consumption rate rapidly decreases and within some hours reaches a low and fairly constant level. The oocyte behaves similarly. At fertilization the rate immediately rises to around the level of the just removed egg, drops slightly and thereafter proceeds after an exponentially increasing curve (cf. 3.21). After natural spawning, fertilization probably occurs very soon, thus leaving the decreasing curve without much importance. Only ripe eggs are fertilizable.

The *Ast.* primary oocyte oxygen consumption rate (Fig. 7b) (cf. 3.122) decreases comparatively slowly in sea water. If the oocyte is ripe it soon starts the first meiosis, thereby increasing its respiration considerably. The second meiosis soon follows and a slow decrease in oxygen consumption continues until fertiliza-

tion occurs. After fertilization the consumption proceeds as an exponentially increasing curve (cf. 3.22). On fertilization no sudden jump in consumption rate occurs as in *Ps.* When at natural spawning the cell leaves the ovary its nuclear membrane has disappeared. Thus the jump in the rate of respiration on transformation from primary to secondary oocyte has already occurred. Even oocytes may be fertilized, but then the time which elapses before the first mitosis will be longer thus allowing meioses to occur and thereafter the resting sperm nucleus to unite with the egg nucleus.

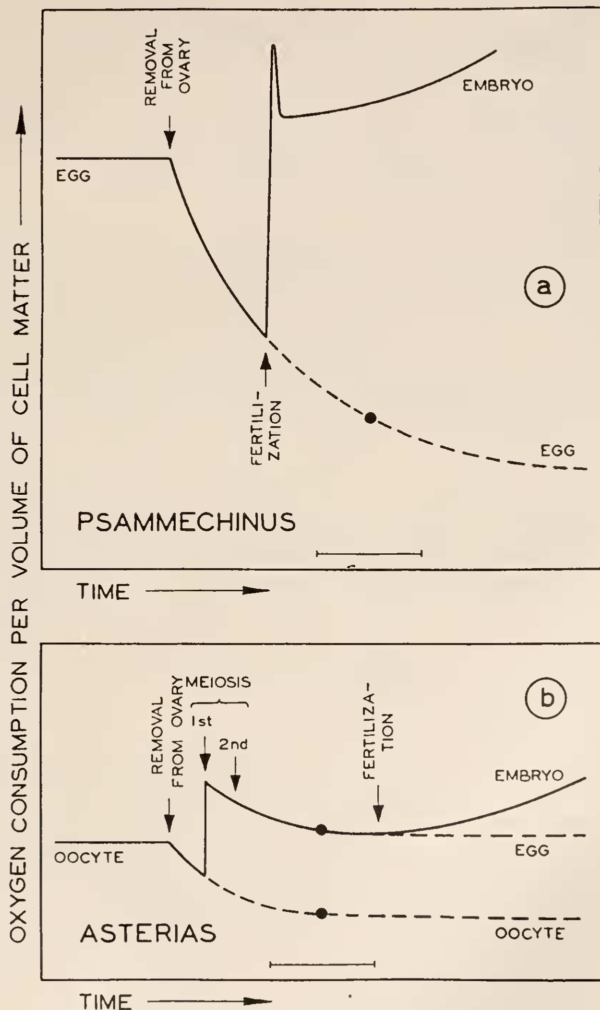

FIGURE 7. Generalized schemes of oxygen consumption per cell volume of reproduction cells of *Psammechinus* and *Asterias*.

Time span of evaluated diver measurements as well as location of comparison value on respiration curve indicated for each cell type.

It is interesting to note that in fertilized eggs of both *Ps.* and *Ast.* the increase in oxygen consumption, which takes place during the first hours of development after fertilization, seems to be of the same exponential type in both species. This will be seen from Figure 8, where values from the first 4 hours' development in both species have been plotted on a relative scale putting the values at 40 minutes after fertilization = 100.

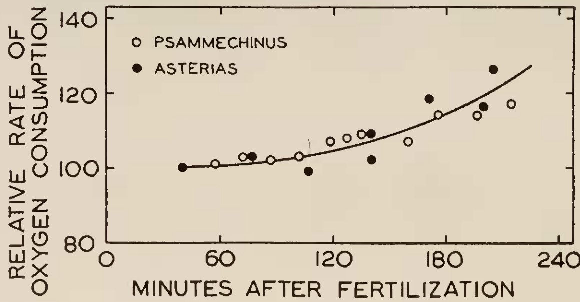

FIGURE 8. Relative oxygen consumption of fertilized *Psammechinus* and *Asterias* eggs. Values at 40 minutes after fertilization put = 100.

5. SUMMARY

In Cartesian diver experiments on the oxygen consumption of oocytes, unfertilized eggs and fertilized eggs from the sea-urchin *Psammechinus miliaris* and the starfish *Asterias glacialis* it was found:

1. The respiration of ripe *Ps.* eggs declines rapidly after they have been removed from the ovary into sea water. Starting at a rate that may exceed that of newly fertilized eggs it has thus, after some hours, attained a comparatively low and fairly constant level. The declining curve on kinetical analysis proves to be composed of a monomolecular and a constant part. The respiration curve of *Ps.* oocytes is of a similar type. In *Ast.* oocytes and eggs the respiratory decrease, though present, is not so prominent as in *Ps.* cells (3.112.1, 3.113, 3.122, Fig. 2).
2. Though there is a real difference in size between the eggs of the two *Ps.* phenotypes (the littoral *Z-form* and the *S-form* of the depths) no difference is found in the rate of respiration (3.112.2, 3.114).
3. Measurements on *Ps.* oocytes and eggs some hours after removal from the ovary show that the oocytes have only a slightly higher respiration than the eggs. The earlier investigations (Lindahl and Holter, 1941) on *Paracentrotus lividus* eggs showed that these oocytes maintain a rate of respiration even higher than that of the newly fertilized egg. The findings in *Par.* might be ascribed to a slow respiration decrease in the oocytes, whereas the decrease is more rapid in the eggs. In *Ps.* the decrease is about equal in oocytes and eggs (3.112.2, 3.113, 4, Fig. 6).
4. In *Ast.* the primary oocytes respire at a much lower rate than do the secondary ones or the eggs (3.122, 4, Fig. 6).
5. In *Ps.* there is a gradual slight decrease in egg respiration with advancing cytoplasmic maturity (3.113).
6. In both *Ps.* and *Ast.* the respiration of oocytes in ovarial fluid seems to be of the same order of magnitude as that of oocytes in sea water (3.113, 3.122).

7. The shape of the respiration curve in *Ps.* after fertilization is in full concordance with earlier results obtained with different techniques by Gray (1926) and Lindahl (1939) (3.21, Fig. 3).

8. The value of the rise in respiration, that occurs in sea-urchin eggs on fertilization, may entirely depend on where on the slope of the decreasing egg respiration curve fertilization occurs. (This rise is characteristic for sea-urchin eggs and has repeatedly been found by earlier investigators.) It is thought that on natural spawning the rise is rather feebly marked because of early fertilization, and that correspondingly the low level respiration of the unfertilized egg may not be reached (3.21, 4, Figs. 3 and 7).

9. In *Ast.* there is no immediate rise in respiration after fertilization, but there is a gradual rise which exactly resembles the exponential increase in newly fertilized sea-urchin eggs (after the first sudden increase has passed). The rise from the oocyte respiration level to that of the egg will, under natural conditions, not occur outside the ovary, as the cells are shed with broken down nuclear membranes (3.22, 4, Figs. 4, 7 and 8).

Cleavage rates are given up to the sixth mitosis for *Ps.*, *Ast.* and *Echinocardium cordatum;* hatching time is noted (3.3, Fig. 5).

It is discussed whether the decrease in respiration of the unfertilized sea-urchin egg after its removal from the ovary has any possible significance for the biochemical aspects of the sea-urchin egg respiration (4).

If the respiration rates found in this investigation are compared on a cell volume basis it is found that the *Ast.* egg will not fit into the generalized scheme of Whitaker (1933) for marine invertebrate eggs; it is discussed why the *Ast.* egg respiration is so comparatively low (4, Fig. 6).

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